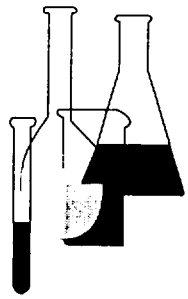
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–98–239
August 1998

EPA

Health Effects Test Guidelines OPPTS 870.6300 Developmental Neurotoxicity Study



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 870.6300 Developmental neurotoxicity study.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP 83–6 Developmental Neurotoxicity Study (Pesticide Assessment Guidelines, Subdivision F--Hazard Evaluation: Human and Domestic Animals, Addendum 10, EPA report 540/09–91–123, March 1991).

(b) **Purpose**. In the assessment and evaluation of the toxic characteristics of a chemical substance or mixture (test substance), determination of the potential for developmental neurotoxicity is important. This study is designed to develop data on the potential functional and morphological hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation.

(c) **Principle of the test method**. The test substance is administered to several groups of pregnant animals during gestation and early lactation, one dose level being used per group. Offspring are randomly selected from within litters for neurotoxicity evaluation. The evaluation includes observations to detect gross neurologic and behavioral abnormalities, determination of motor activity, response to auditory startle, assessment of learning, neuropathological evaluation, and brain weights. This protocol may be used as a separate study, as a followup to a standard developmental toxicity and/or adult neurotoxicity study, or as part of a two-generation reproduction study, with assessment of the offspring conducted on the second (F_2) generation.

(d) **Test procedure**—(1) **Animal selection**—(i) **Species and strain.** Testing should be performed in the rat. Because of its differences in timing of developmental events compared to strains that are more commonly tested in other developmental and reproductive toxicity studies, it is preferred that the Fischer 344 strain not be used. If a sponsor wishes to use the Fischer 344 rat or a mammalian species other than the rat, ample justification/reasoning for this selection must be provided.

(ii) **Age.** Young adult (nulliparous females) animals should be used.

(iii) **Sex.** Pregnant female animals should be used at each dose level.

(iv) **Number of animals.** (A) The objective is for a sufficient number of pregnant rats to be exposed to the test substance to ensure that an adequate number of offspring are produced for neurotoxicity evaluation. At least 20 litters are recommended at each dose level.

(B) On postnatal day 4, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, four male and four females per litter. Whenever the number of pups of either sex prevents having four of each sex per litter, partial adjustment (for example, five males and three females) is permitted. Testing is not appropriate for litters of less than seven pups. Elimination of runts only is not appropriate. Individual pups should be identified uniquely after standardization of litters. A method that may be used for identification can be found under paragraph (f)(1) of this guideline.

(v) **Assignment of animals for behavioral tests, brain weights, and neuropathological evaluations.** After standardization of litters, one male or one female from each litter (total of 10 males and 10 females per dose group) should be randomly assigned to one of the following tests: Motor activity, auditory startle, and learning and memory, in weanling and adult animals. On postnatal day 11, either 1 male or 1 female pup from each litter (total of 10 males and 10 females per dose group) should be sacrificed. Brain weights should be measured in all of these pups and, of these pups, six per sex per dose should be selected for neuropathological evaluation. At the termination of the study, either 1 male or 1 female from each litter (total of 10 males and 10 females per dose group) should be sacrificed and brain weights should be measured. An additional group of six animals per sex per dose group (one male or one female per litter) should be sacrificed at the termination of the study for neuropathological evaluation.

(2) **Control group**. A concurrent control group is required. This group should be a sham-treated group or, if a vehicle is used in administering the test substance, a vehicle control group. The vehicle should neither be developmentally toxic nor have effects on reproduction. Animals in the control group should be handled in an identical manner to test group animals.

(3) **Dose levels and dose selection.** (i) At least three dose levels of the test substance plus a control group (vehicle control, if a vehicle is used) should be used.

(ii) If the test substance has been shown to be developmentally toxic either in a standard developmental toxicity study or in a pilot study, the highest dose level should be the maximum dose which will not induce in utero or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity.

(iii) If a standard developmental toxicity study has not been conducted, the highest dose level, unless limited by the physicochemical nature or biological properties of the substance, should induce some overt maternal toxicity, but should not result in a reduction in weight gain exceeding 20 percent during gestation and lactation.

(iv) The lowest dose should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate doses should be equally spaced between the highest and lowest doses used.

(4) **Dosing period.** Day 0 of gestation is the day on which a vaginal plug and/or sperm are observed. The dosing period should cover the period from day 6 of gestation through day-10 postnatally. Dosing should not occur on the day of parturition in those animals who have not completely delivered their offspring.

(5) **Administration of the test substance**. The test substance or vehicle should be administered orally. Other routes of administration may be acceptable, on a case-by-case basis, with ample justification/reasoning for this selection. The test substance or vehicle should be administered based on the most recent weight determination.

(6) **Observation of dams**. (i) A gross examination of the dams should be made at least once each day before daily treatment.

(ii) Ten dams per group should be observed outside the home cage at least twice during the gestational dosing period (days 6-21) and twice during the lactational dosing period (days 1-10) for signs of toxicity. The animals should be observed by trained technicians who are unaware of the animals' treatment, using standardized procedures to maximize interobserver reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of interobserver reliability is required.

(iii) During the treatment and observation periods under paragraph (d)(6)(ii), observations should include:

(A) Assessment of signs of autonomic function, including but not limited to:

(*1*) Ranking of the degree of lacrimation and salivation, with a range of severity scores from none to severe.

(*2*) Presence or absence of piloerection and exophthalmus.

(*3*) Ranking or count of urination and defecation, including polyuria and diarrhea.

(*4*) Pupillary function such as constriction of the pupil in responseto light or a measure of pupil size.

(*5*) Degree of palpebral closure, e.g., ptosis.

(B) Description, incidence, and severity of any convulsions, tremors, or abnormal movements.

(C) Description and incidence of posture and gait abnormalities.

(D) Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

(iv) Signs of toxicity should be recorded as they are observed, including the time of onset, degree, and duration.

(v) Animals should be weighed at least weekly and on the day of delivery and postnatal days 11 and 21 (weaning) and such weights should be recorded.

(vi) The day of delivery of litters should be recorded and considered as postnatal day 0.

(7) **Study conduct—(i) Observation of offspring.** (A) All offspring should be examined cage-side at least daily for gross signs of mortality or morbidity.

(B) A total of 10 male offspring and 10 female offspring per dose group should be examined outside the cage for signs of toxicity on days 4, 11, 21, 35, 45, and 60. The offspring should be observed by trained technicians, who are unaware of the treatment being used, using standardized procedures to maximize interobserver reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of interobserver reliability is required. At a minimum, the end points outlined in paragraph (d)(6)(iii) of this guideline should be monitored as appropriate for the developmental stage being observed.

(C) Any gross signs of toxicity in the offspring should be recorded as they are observed, including the time of onset, degree, and duration.

(ii) **Developmental landmarks.** Live pups should be counted and each pup within a litter should be weighed individually at birth or soon thereafter, and on postnatal days 4, 11, 17, and 21 and at least once every 2 weeks thereafter. The age of vaginal opening and preputial separation should be determined. General procedures for these determinations may be found in paragraphs (f)(1) and (f)(11) of this guideline.

(iii) **Motor activity.** Motor activity should be monitored specifically on postnatal days 13, 17 21, and 60 (±2 days). Motor activity must be monitored by an automated activity recording apparatus. The device must be capable of detecting both increases and decreases in activity, (i.e., baseline activity as measured by the device must not be so low as to preclude detection of decreases nor so high as to preclude detection of increases in activity). Each device should be tested by standard procedures to ensure,

to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices. Each animal should be tested individually. The test session should be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for nontreated control animals. All sessions should have the same duration. Treatment groups should be counter-balanced across test times. Activity counts should be collected in equal time periods of no greater than 10 minutes duration. Efforts should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, light conditions, odors, use of home cage or novel test cage, and environmental distractions. Additional information on the conduct of a motor activity study may be obtained in OPPTS 870.6200.

(iv) **Auditory startle test.** An auditory startle habituation test should be performed on the offspring around the time of weaning and around day 60. Day of testing should be counterbalanced across treated and control groups. Details on the conduct of this testing may be obtained under paragraph (f)(1) of this guideline. In performing the auditory startle task, the mean response amplitude on each block of 10 trials (5 blocks of 10 trials per session on each day of testing) should be made. While use of prepulse inhibition is not a requirement, it is highly recommended. Details on the conduct of this test may be obtained in paragraph (f)(10) of this guideline

(v) **Learning and memory tests.** A test of associative learning and memory should be conducted around the time of weaning and around day 60. Day of testing should be counterbalanced across treated and control groups. The same or separate tests may be used at these two stages of development. Some flexibility is allowed in the choice of tests for learning and memory in weanling and adult rats. However, the tests must be designed to fulfill two criteria. First, learning must be assessed either as a change across several repeated learning trials or sessions, or, in tests involving a single trial, with reference to a condition that controls for non-associative effects of the training experience. Second, the tests should include some measure of memory (short-term or long-term) in addition to original learning (acquisition). If the tests of learning and memory reveal an effect of the test compound, it may be in the best interest of the sponsor to conduct additional tests to rule out alternative interpretations based on alterations in sensory, motivational, and/or motor capacities. In addition to the above two criteria, it is recommended that the test of learning and memory be chosen on the basis of its demonstrated sensitivity to the class of compound under investigation, if such information is available in the literature. In the absence of such information, examples of tests that could be made to meet the above criteria include: Delayed-matching-to-position, as described for the adult rat (see paragraph (f)(3) of this guideline) and

for the infant rat (see paragraph (f)(9) of this guideline); olfactory conditioning, as described in paragraph (f)(13) of this guideline; and acquisition and retention of schedule-controlled behavior (see paragraphs (f)(4) and (f)(5) of this guideline). Additional tests for weanling rats are described under paragraphs (f)(20) and (f)(12) of this guideline, and for adult rats under paragraph (f)(16) of this guideline.

(vi) **Neuropathology.** Neuropathological evaluation should be conducted on animals on postnatal day 11 and at the termination of the study. At 11 days of age, one male or female pup should be removed from each litter such that equal numbers of male and female offspring are removed from all litters combined. Of these, six male and six female pups per dose group will be sacrificed for neuropathological analysis. The pups will be killed by exposure to carbon dioxide and immediately thereafter the brains should be removed, weighed, and immersion-fixed in an appropriate aldehyde fixative. The remaining animals will be sacrificed in a similar manner and immediately thereafter their brains removed and weighed. At the termination of the study, one male or one female from each litter will be killed by exposure to carbon dioxide and immediately thereafter the brain should be removed and weighed. In addition, six animals per sex per dose group (one male or female per litter) should be sacrificed at the termination of the study for neuropathological evaluation. Neuropathological analysis of animals sacrificed at the termination of the study should be performed in accordance with OPPTS 870.6200. Neuropathological evaluation of animals sacrificed on postnatal day 11 and at termination of the study should include a qualitative analysis and semiquantitative analysis as well as simple morphometrics.

(A) **Fixation and processing of tissue samples for postnatal day 11 animals.** Immediately following removal, the brain should be weighed and immersion fixed in an appropriate aldehyde fixative. The brains should be postfixed and processed according to standardized published histological protocols under paragraphs (f)(6), (f)(14), (f)(17), and (f)(21) of this guideline. Paraffin embedding is acceptable but plastic embedding is preferred and recommended. Tissue blocks and slides should be appropriately identified when stored. Histological sections should be stained for hematoxylin and eosin, or a similar stain according to standard published protocols under paragraphs (f)(2), (f)(18), and (f)(23) of this guideline. For animals sacrificed at the termination of the study, methods for fixation and processing of tissue samples are provided in paragraph (e)(4)(iv)(A) of OPPTS 870.6200.

(B) **Qualitative analysis.** The purposes of the qualitative examination are threefold—to identify regions within the nervous system exhibiting evidence of neuropathological alterations, to identify types of neuropathological alterations resulting from exposure to the test substance, and to determine the range of severity of the neuropathological alterations. Representative histological sections from the tissue samples should be examined mi-

croscopically by an appropriately trained pathologist for evidence of neuropathological alterations. The following stepwise procedure is recommended for the qualitative analysis. First, sections from the high dose group are compared with those of the control group. If no evidence of neuropathological alterations is found in animals of the high dose group, no further analysis is required. If evidence of neuropathological alterations are found in the high dose group, then animals from the intermediate and low dose group are examined. Subject to professional judgment and the kind of neuropathological alterations observed, it is recommended that additional methods such as Bodian's or Bielchowsky's silver methods and/or immunohistochemistry for glial fibrillary acid protein be used in conjunction with more standard stains to determine the lowest dose level at which neuropathological alterations are observed. Evaluations of postnatal day 11 pups is described in paragraphs (d)(7)(vi)(B)(*1*) and (d)(7)(vi)(B)(*2*) of this guideline. For animals sacrificed at the termination of the study, the regions to be examined and the types of alterations that should be assessed are identified in paragraph (e)(4)(iv)(B) of OPPTS 870.6200.

(*1*) **Regions to be examined.** The brains should be examined for any evidence of treatment-related neuropathological alterations and adequate samples should be taken from all major brain regions (e.g., olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain (tectum, tegmentum, and cerebral peduncles), brainstem and cerebellum) to ensure a thorough examination.

(*2*) **Types of alterations**. Guidance for neuropathological examination for indications of developmental insult to the brain can be found in paragraphs (f)(8) and (f)(22) of this guideline. In addition to more typical kinds of cellular alterations (e.g., neuronal vacuolation, degeneration, necrosis) and tissue changes (e.g., astrocytic proliferation, leukocytic infiltration, and cystic formation) particular emphasis should be paid to structural changes indicative of developmental insult including but not restricted to:

(*i*) Gross changes in the size or shape of brain regions such as alterations in the size of the cerebral hemispheres or the normal pattern of foliation of the cerebellum.

(*ii*) The death of neuronal precursors, abnormal proliferation, or abnormal migration, as indicated by pyknotic cells or ectopic neurons, or gross alterations in regions with active proliferative and migratory zones, alterations in transient developmental structures (e.g., the external germinal zone of the cerebellum, see paragraph (f)(15) of this guideline).

(*iii*) Abnormal differentiation, while more apparent with special stains, may also be indicated by shrunken and malformed cell bodies.

(*iv*) Evidence of hydrocephalus, in particular enlargement of the ventricles, stenosis of the cerebral aqueduct and general thinning of the cerebral hemispheres.

(C) **Subjective diagnosis.** If any evidence of neuropathological alterations is found in the qualitative examination, then a subjective diagnosis will be performed for the purpose of evaluating dose-response relationships. All regions of the brain exhibiting any evidence of neuropathological changes should be included in this analysis. Sections of each region from all dose groups will be coded as to treatment and examined in randomized order. The frequency of each type and the severity of each lesion will be recorded. After all sections from all dose groups including all regions have been rated, the code will be broken and statistical analyses performed to evaluate dose-response relationships. For each type of dose related lesion observed, examples of different ranges of severity should be described. The examples will serve to illustrate a rating scale, such as 1+, 2+, and 3+ for the degree of severity ranging from very slight to veryextensive.

(D) **Simple morphometric analysis.** Since the disruption of developmental processes is sometimes more clearly reflected in the rate or extent of growth of particular brain regions, some form of morphometric analysis should be performed on postnatal day 11 and at the termination of the study to assess the structural development of the brain. At a minimum, this would consist of a reliable estimate of the thickness of major layers at representative locations within the neocortex, hippocampus, and cerebellum. For guidance on such measurements see Rodier and Gramann under paragraph (f)(19) of this guideline.

(e) **Data collection, reporting, and evaluation.** The following specific information should be reported:

(1) **Description of test system and test methods.** A description of the general design of the experiment should be provided. This should include:

(i) A detailed description of the procedures used to standardize observations and procedures as well as operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. These data do not have to be from studies using prenatal exposures. However, the laboratory must demonstrate competence in evaluation effects in neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group.

(iii) Procedures for calibrating and ensuring the equivalence of devices and the balancing of treatment groups in testing procedures.

(iv) A short justification explaining any decisions involving professional judgement.

(2) **Results.** The following information must be arranged by each treatment and control group:

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number and the litter from which it came.

(B) Its body weight and score on each developmental landmark at each observation time.

(C) Total session activity counts and intrasession subtotals on each day measured.

(D) Auditory startle response amplitude per session and intrasession amplitudes on each day measured.

(E) Appropriate data for each repeated trial (or session) showing acquisition and retention scores on the tests of learning and memory on each day measured.

(F) Time and cause of death (if appropriate); any neurological signs observed; a list of structures examined as well as the locations, nature, frequency, and extent of lesions; and brain weights.

(ii) The following data should also be provided, as appropriate:

(A) Inclusion of photomicrographs demonstrating typical examples of the type and extent of the neuropathological alterations observed is recommended.

(B) Any diagnoses derived from neurological signs and lesions, including naturally occurring diseases or conditions, should also be recorded.

(iii) Summary data for each treatment and control group must include:

(A) The number of animals at the start of the test.

(B) The body weight of the dams during gestation and lactation.

(C) Litter size and mean weight at birth.

(D) The number of animals showing each abnormal sign at each observation time.

(E) The percentage of animals showing each abnormal sign at each observation time.

(F) The mean and standard deviation for each continuous endpoint at each observation time. These will include body weight, motor activity counts, auditory startle responses, performance in learning and memory tests, regional brain weights and whole brain weights (both absolute and relative).

(G) The number of animals in which any lesion was found.

(H) The number of animals affected by each different type of lesion, the location, frequency and average grade of each type of lesion for each animal.

(I) The values of all morphometric measurements made for each animal listed by treatment group.

(3) **Evaluation of data.** An evaluation of test results must be made. The evaluation should include the relationship between the doses of the test substance and the presence or absence, incidence, and extent of any neurotoxic effect. The evaluation should include appropriate statistical analyses. The choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons. The evaluation should include the relationship, if any, between observed neuropathological and behavioral alterations.

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Adams, J., Buelke-Sam, J., Kimmel, C.A., Nelson, C.J., Reiter, L.W., Sobotka, T.J., Tilson, H. A., and Nelson, B.K. Collaborative behavioral teratolgy study: Protocol design and testing procedures. *Neurobehavioral Toxicology and Teratology* 7:579–586 (1985).

(2) Bennett, H.S., Wyrick, A.D., Lee, S.W., and McNeil, J.H. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technology* 51:71–97 (1976).

(3) Bushnell, P.J. Effects of delay, intertrial interval, delay behavior and trimethyltin on spatial delayed response in rats. *Neurotoxicology and Teratology* 10:237–244 (1988).

(4) Campbell, B.A. and Haroutunian, V. Effects of age on long-term memory:Retention of fixed interval responding. *Journal of Gerontology* 36:338–341 (1981).

(5) Cory-Slechta, D.A., Weiss, B., and Cox, C. Delayed behavioral toxicity of lead with increasing exposure concentration. *Toxicology and Applied Pharmacology* 71:342–352 (1983).

(6) Di Sant Agnese, P. A. and De Mesy Jensen, K.L. Dibasic staining of large epoxy tissue sections and application to surgical pathology. *American Journal of Clinical Pathology* 81:25–29 (1984).

(7) U.S. Environmental Protection Agency. Neurotoxicity Screening Battery. In: Pesticide Assessment Guidelines, Subdivision F, Addendum 10. EPA 540/09-91–123. NTIS PB 91–154617. (1991).

(8) Friede, R. L. *Developmental Neuropathology.* Springer-Verlag, New York. pp. 1–23, 297–313, 326–351. (1975).

(9) Green, R.J. and Stanton, M.E. Differential ontogeny of working memory and reference memory in the rat. *Behavioral Neuroscience* 103:98–105 (1989).

(10) Ison, J.R. Reflex modification as an objective test for sensory processing following toxicant exposure. *Neurobehavioral Toxicology and Teratology* 6:437–445 (1984).

(11) Korenbrot, C.C., Huhtaniemi, I.T., and Weiner, R.I. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* 17:298–303 (1977).

(12) Krasnegor, N.A., Blass, E.M., Hofer, M.A., and Smotherman, W.P. (eds.) *Perinatal Development: A Psychobiological Perspective.* Academic Press, Orlando. pp. 11–37, 145–167. (1987).

(13) Kucharski, D. and Spear, N.E. Conditioning of aversion to an odor paired with peripheral shock in the developing rat. *Developmental Psychobiology* 17:465–479 (1984).

(14) Luna, L. G. *(editor). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* (Third Edition). McGraw-Hill, New York. pp. 1–31. (1968).

(15) Miale, I. L. and Sidman, R.L. An autoradiographic analysis of histogenesis in the mouse cerebellum. *Experimental Neurology.* 4:277–296 (1961).

(16) Miller, D.B. and Eckerman, D.A. Learning and memory measures. In: *Neurobehavioral Toxicology* , Z. Annau (ed). Johns Hopkins University Press, Baltimore. pp. 94–149 (1986).

(17) Pender, M.P. A simple method for high resolution light microscopy of nervous tissue. *Journal of Neuroscience Methods.* 15:213–218 (1985).

(18) Ralis, H.M., Beesley, R.A., and Ralis, Z.A. *Techniques in Neurohistology.* Butterworths, London. pp. 57–145. (1973).

(19) Rodier, P.M. and Gramann, W.J. Morphologic effects of interference with cell proliferation in the early fetal period. *Neurobehavioral Toxicology* 1:129–135 (1979).

(20) Spear, N.E. and Campbell, B.A. (eds.) *Ontogeny of Learning and Memory.* Erlbaum, New Jersey. pp. 101–133, 157–224. (1979).

(21) Spencer, P.S., Bischoff, M.C., and Schaumburg, H.H. Neuropathological methods for the detection of neurotoxic disease. In: Ex-

perimental and Clinical Neurotoxicology. Spencer, P.S. and Schaumburg, H.H. (eds.). Williams and Wilkins, Baltimore. pp. 743–757. (1980)

(22) Suzuki, K. Special vulnerabilities of the developing nervous system to toxic substances. In: *Experimental and Clinical Neurotoxicology.* Spencer, P.S. and Schaumburg, H.H. (eds.). Williams and Wilkins, Baltimore. pp. 48–61 (1980).

(23) Luna, L.G. (Editor). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. (Third Edition). McGraw-Hill, New York. pp. 32–46. (1968).